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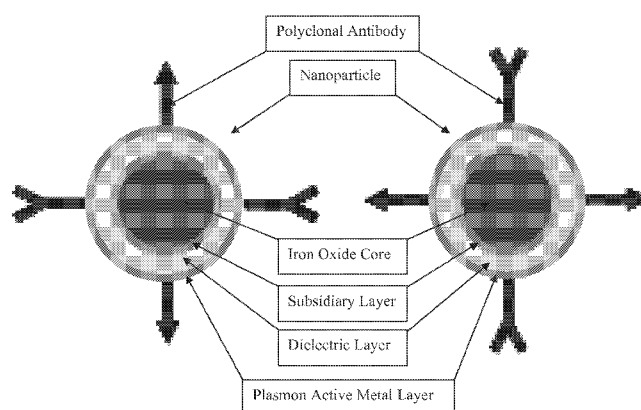


FIG. 1

(57) Abstract: Compositions and methods for detecting the presence or concentration of a target antigen in a solution. Compositions include a plurality of nanoparticles, each comprising a core comprising iron oxide, and a shell comprising at least one plasmon active metal, where an exterior surface of each nanoparticle is functionalized with a plurality of polyclonal antibodies, each independently configured to bind to at least a portion of the target antigen. Methods include preparing a solution comprising the composition and the target antigen, where at least some of the plurality of nanoparticles bind to the target antigen to form a nanoparticle-antigen aggregate. The nanoparticles that form the nanoparticle-antigen aggregate absorb electromagnetic radiation differently from nanoparticles that do not form the nanoparticle-antigen aggregate.



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DETECTING ANTIGENS SUCH AS BACTERIAL QUORUM SENSING PROTEINS**Introduction**

Monitoring the course of bacterial infection – especially antibiotic resistant strains – is paramount in improving patient outcomes and decreasing communal disease persistence. Unfortunately, means of monitoring infection progression are laboratory-based and temporally inefficient.

Bacterial infections are generally diagnosed by obtaining specimen samples from patients, extracting bacterium from the specimen samples, culturing the bacterium in the laboratory, and performing bacterial identification via performing genotypic characterization using polymerase chain reaction (PCR) strategies. This general strategy is disadvantageous for a number of reasons. First, obtaining bacteria cells via biopsy from patient specimen can become surgically-invasive. Further, the time necessary to perform the specimen purification, cell culturing and genotypic characterization could take 4-7 days (non-rapid, temporally inefficient). These procedures must also be performed in a laboratory (non-portable, not point-of-care, specialized training a requirement). Other attempts at developing point-of-care strategies for bacterial diagnostics are limited because they require bacterial cell surfaces and/or intracellular constituents for identification – again possibly requiring invasive methods for obtaining such a specimen.

Summary

This disclosure provides compositions for detecting the presence or concentration of a target antigen, comprising a plurality of nanoparticles. Each nanoparticle comprises a core comprising iron oxide, and a shell comprising at least

one plasmon active metal, where an exterior surface of each nanoparticle is functionalized with a plurality of polyclonal antibodies, each independently configured to bind to at least a portion of the target antigen. When a solution is prepared comprising the composition and the target antigen, at least some of the plurality of nanoparticles bind to the target antigen to form a nanoparticle-antigen aggregate. The nanoparticles that form the nanoparticle-antigen aggregate absorb electromagnetic radiation differently from nanoparticles that do not form the nanoparticle-antigen aggregate.

This disclosure also provides methods of detecting the presence or concentration of a target antigen. The method comprises preparing a solution comprising the target antigen with a composition comprising a plurality of nanoparticles. Each nanoparticle comprises a core comprising iron oxide, and a shell comprising at least one plasmon active metal. An exterior surface of each nanoparticle is functionalized with a plurality of polyclonal antibodies, each independently configured to bind to at least a portion of the target antigen. When the solution is prepared, at least some of the plurality of nanoparticles bind to the target antigen to form a nanoparticle-antigen aggregate. The method further comprises measuring a change in the absorbance spectra of the solution.

Brief Description of the Drawings

FIG. 1 is a schematic showing an exemplary composition comprising a plurality of nanoparticles, according to aspects of this disclosure.

FIG. 2 is an illustration showing contacting the composition of FIG. 1 with a solution comprising a bacterial quorum sensing protein.

FIG. 3 is an illustration showing nanoparticles binding to bacterial quorum sensing proteins to form an aggregate.

FIG. 4 is an illustration showing a plurality of aggregates.

Detailed Description

This disclosure provides compositions, devices and methods for detecting the presence or concentration of target antigens, such as bacterial quorum sensing proteins.

The compositions, devices and methods may be used, for example, to diagnose and/or monitor the progression of a bacterial infection using easily obtainable, low-volume, patient fluid samples (blood, saliva, sputum, mucus, fecal matter, genital secretions). The concentration of the target antigen, such as a bacterial quorum sensing protein, may be directly related to other systemic indications, such as the cell mass of a bacteria population during an infection. Using this correlation, the progression of an infection – both before and after treatment – can be monitored in a point-of-care, personalized fashion.

This compositions, devices and methods may provide for a rapid (<20 min), single-step, sensitive, solution-phase, antigen detection method based on intermolecular recognition nanoparticulate self-assembly. Briefly, photonicallly-active, magnetic nanoparticles with a constant peak absorbance will be conjugated with polyvalent antibodies against the target antigen. Following incubation with a solution containing, the nanoparticles bind with the target antigen thereby causing the nanoparticles to aggregate into self-assembled structures. This 'programmed-agglomeration' may cause a change in the absorbance properties of the nanoparticles –

thus providing a detectable property that is directly correlated to the presence and concentration of the target antigen. In the case of targeting bacterial quorum sensing proteins in solution, detecting the presence and concentration of the protein may allow for the determination of the approximate cell mass of a bacteria population. The nanoparticles used for this method have absorption peaks outside of the biologically-sensitive, visible wavelength range, thus allowing for easy analysis of biological samples (e.g. blood, saliva, sputum, etc.).

The description below, with reference to FIGS. 1-4 provides additional details of the methods, devices and compositions of this disclosure:

Compositions for detecting the presence of a target antigen.

Fig. 1 shows an exemplary embodiment of a composition for detecting the presence of a target antigen. The composition comprises a superparamagnetic iron-oxide core (e.g., Fe_2O_3 or Fe_3O_4), an optional first shell formed of a dielectric material (e.g., SiO_2 , among others) surrounding the core, and an outer shell formed of one or more plasmon active metals (e.g., gold, silver, copper, platinum). Depending on the overall conformation of these nanoparticles, they may be configured to absorb electromagnetic radiation at a first peak wavelength, such as a wavelength in the UV, visible, infrared (IR) and near-infrared (NIR) regions of the electromagnetic spectrum.

In order to provide nanoparticles which have so many functional layers, each shell must be carefully added through controlled fabrication methods. These methods permit the fabrication of extremely thin shells (as small as 1-2 nm to maintain an overall particle diameter less than about 100nm, such as less than about 90nm, less than about 80nm, less than about 70nm and preferably, less than about 60 nm) while still ensuring

magnetic material retention throughout the fabrication process. In some embodiments, these methods may include coating a superparamagnetic iron oxide particle (e.g., Fe_2O_3 or Fe_3O_4) with a first aminosilane (e.g., APTES, APTMS, APDEMS, APEMS, etc.) to form an aminated core, coating the aminated core with a first shell formed of a dielectric material (e.g., SiO_2 , among others) using sonication, coating the first shell with a second aminosilane (e.g., APTES, APTMS, APDEMS, or APEMS, a cyclic aminosilane such as *N-n*-butyl-aza-2,2-dimethoxysilacyclopentane, etc.) to form an aminated first shell, and coating the aminated first shell with a second shell formed of one or more plasmon active metals (e.g., gold, silver, copper, platinum). In some embodiments, the dielectric material may be added directly to the iron oxide core without the use of a first aminosilane layer. In some embodiments, the iron oxide core may be directly coated with the one or more plasmon active metals.

In order to detect the presence or concentration of antigens (e.g., bacterial quorum sensing proteins (QSPs), or any other desired antigen) using these nanoparticles as bionanoprobes, their outer surface may be functionalized with polyvalent antibodies raised against the target antigen. Polyvalent antibodies may be utilized (as opposed to monoclonal antibodies) due to their ability to bind to different structures or motifs on the surface of the target antigen. Specifically, the exterior surface of one or more (i.e., at least one) nanoparticles may be functionalized by at least a first antibody configured to bind to a first portion of the target antigen, and a second antibody configured to bind to a second portion of the target antigen that is different from the first portion. This allows a single antigen to simultaneously be agglutinated with more than one antibody (and thus more than one nanoparticle). The

technique disclosed herein leverages this ability by encouraging the formation of nanoparticle-antigen aggregates, which also may be referred to as “immuno-agglomerates”. Specifically, as shown in Figs. 2-4, when a solution is prepared comprising a plurality of nanoparticles and a target antigen, at least some of the plurality of nanoparticles may bind to the target antigen to form a nanoparticle-antigen aggregate.

The polyclonal antibodies may be attached or otherwise conjugated to the surface of the nanoparticles using any of a number of different methods. For example, a heterobifunctional polyethylene glycol (PEG) linker may be used, such as OPSS-PEG-NHS ((ortho-pyridyl)disulfide-PEG-Succinimidyl Ester), where the OPSS sulfur groups may bind strongly to the gold surface, and the NHS groups may form an amide linkage the PEG to amine containing residues on the antibodies, thereby forming an amide linkage. Alternatively or additionally, a streptavidin group may be conjugated to the exterior surface of the gold shell (e.g., using a glycine-saline buffer solution of commercially procured streptavidin molecules) thus allowing for functionalization of the surface with biotinylated-polyclonal antibodies. A plethora of other functionalization strategies may be utilized by those skilled in the art.

The nanoparticles of this disclosure may be configured to form nanoparticle-antigen aggregates wherein the aggregated nanoparticles absorb electromagnetic radiation differently from the free nanoparticles. For example, whereas the nanoparticles that do not form a nanoparticle-antigen aggregate may absorb electromagnetic radiation at a first peak wavelength, the nanoparticles that form the nanoparticle-antigen aggregates may absorb electromagnetic radiation at a second

peak wavelength different from the first peak wavelength, such as a wavelength in the UV, visible, infrared (IR) and near-infrared (NIR) regions of the electromagnetic spectrum. In some embodiments, the first peak wavelength may be less than the second peak wavelength. Alternatively or additionally, the nanoparticles that do not form nanoparticle-antigen aggregate may have a molar extinction coefficient at a selected wavelength (e.g., the first peak wavelength or another selected wavelength) that is different from the molar extinction coefficient at the selected wavelength of the nanoparticles that form the nanoparticle-antigen aggregate.

The nanoparticles of this disclosure also may be configured to form nanoparticle-antigen aggregates wherein the aggregated nanoparticles have magnetic properties different from the free nanoparticles. For example, the nanoparticles that do not form the nanoparticle-antigen aggregate may have a first magnetic relaxation time, and the nanoparticles that form the nanoparticle-antigen aggregate may have a second magnetic relaxation time different from the first magnetic relaxation time.

Methods for detecting the presence or concentration of a target antigen.

Methods of using the nanoparticles may include preparing a solution comprising the target antigen and a composition comprising the nanoparticles discussed above. When the solution is prepared, the polyclonal antibodies functionalized on the surfaces of the nanoparticles bind to the target antigen. This allows each antigen to be agglutinated with one or more nanoparticles, thus causing the nanoparticles to form nanoparticle-antigen agglomerates. After a number of self-assembly driven aggregation events, the nanoparticle-antigen aggregates may form particles growing to diameters larger than 1 μm (Fig. 4).

As discussed above, the nanoparticles in these aggregates may have different extinction properties with respect to the absorbance of electromagnetic radiation, and different magnetic properties relative to the free nanoparticles. For example, in some cases, a slight shifting of the extinction peak may occur from a first peak wavelength to a second peak wavelength upon aggregation. Monitoring the change in absorption at the first and/or the second wavelengths may allow for the detection of the presence or concentration (e.g., via the use of a standard curve) of the target antigen. Moreover, a change in the magnetic properties (e.g., the magnetic relaxation time) of the solution also may indicate the formation of immuno-agglomerates and thus permit the detection of target antigens as well. As such, some methods may include the step of measuring a change in the absorbance spectra of the solution, such as measuring a change at the first peak wavelength, the second peak wavelength or both, where the relative amount of the measured absorbance change may be proportional to the concentration of the antigen in the solution. Some methods may include the step of measuring a change in the magnetic relaxation time of the solution.

In the case of target bacterial quorum proteins, a change in extinction characteristics and/or magnetic properties may be directly related to the concordant bacterial quorum protein concentration and thus the bacterial cell load during an infection. As such, the compositions and methods of this disclosure do not require the presence of actual bacterium for diagnosis of an infection. Moreover, the methods may be performed very quickly and efficiently without the need for culturing samples in a lab, or conducting time-consuming PCR experiments.

Solutions comprising a target antigen may include, but are not limited to, biological samples taken from a subject, *in vitro* culture extractions, etc. Exemplary samples may include, but are not limited to, blood, saliva, sputum, urine, fecal matter, seminal fluid, vaginal fluid, and tissue samples. Solutions comprising the target antigen therefore may include non-target biological molecules (e.g., proteins, DNA, lipids, etc.) that are capable of absorbing light in the UV and visible regions of the electromagnetic spectrum. In order to make sure that non-target biological molecules do not interfere with the detection of changes in either the first or second wavelengths, the peak absorbance of either the non-aggregated (i.e., free) nanoparticles, the nanoparticle-antigen aggregates, or both, may be in the IR or NIR regions rather than the UV-visible regions of the electromagnetic spectrum. Any absorption-based assays utilizing UV and visible regions necessarily would be inhibited by the background absorbance of non-target biological components.

A device may be used in conjunction with the compositions disclosed herein. For example, the device may include a reader and a cartridge or cassette. The reader may include a spectrophotometer and a receiver for receiving the cartridge or cassette. The cartridge or cassette may include a sample reservoir loaded with the compositions disclosed herein, and having a volume for receiving a sample that potentially comprises the target antigen. In some embodiments, the device may function as a cassette reader for 'one-use' cartridges. Different cartridges may be loaded with different compositions configured to detect the presence or concentration of different target antigens, such as different bacterial quorum sensing proteins associated with different bacteria strains. The devices and methods disclosed herein may be used to analyze samples from a

patient several times a day, so as to monitor the course or duration of an infection. The device may be portable so as to permit use at essentially any point of care.

EXAMPLES

Fabrication of non-functionalized nanoparticles

FeOx-SiO₂-Au nanoparticles (specifically, Fe₂O₃-SiO₂-Au nanoparticles) were fabricated by performing the following protocol.

First, 12 ±1 nm diameter γ-Fe₂O₃ nanoparticles were fabricated by a thermal decomposition, aeration, and reflux protocol. Briefly, octyl ether (Sigma-Aldrich, St. Louis, MO, 20 mL) and oleic acid (Sigma-Aldrich, 1.92 mL) were stirred under N₂ gas flow and reflux. The sample was heated to 100°C prior to addition of Fe(CO)₅ (Sigma-Aldrich, 0.4 mL). The reaction was heated from 150°C to 280°C, where the reaction solution color changed from boil to orange, orange/colorless, to very dark orange. The reaction was aerated at 80°C for 14 hours and refluxed while boiling for 2 hours. γ-Fe₂O₃ cores were then centrifuged (15 min, 770 relative centrifugal force (rcf)) and washed in ethanol (EtOH, 200 proof, Sigma-Aldrich) thrice, and dried under air.

The FeOx nanoparticles (150 μL, 0.2% concentration) were subsequently coated with a silica layer by adding EtOH (200 proof, 4.85 μL), ethanolic TEOS (Sigma-Aldrich, 1 M, 10 μL) and 40% NH₄OH (35 μL) under ultrasonic perturbation for 2 hours at room temperature. Resultant particles were stored thereafter at 4°C for 24 hours. FeOx-SiO₂ nanoparticles were coated with cyclic silane (Gelest, SIB1932.4), where 1 mM ethanolic cyclic silane (400 μL) was added under ultrasonic perturbation to the ethanolic suspension of FeOx-SiO₂ nanoparticles. The solution of FeOx-SiO₂-NH₂ nanoparticles was stored at 4°C for 24 hours where they remained stable until completely utilized.

FeOx-SiO₂-NH₂ nanoparticles were decorated through emersion in Duff gold colloid (2–4 nm, dark-aged for 3 weeks in 4°C) in a 1:4, particle to colloid ratio. Briefly, FeOx-SiO₂-NH₂ nanoparticles (~5 mL) was mixed with Duff Au colloid (20 mL). This mixture was left unperturbed at room temperature (20–23°C) for 24–168 hours, centrifuged (10 min, 800 rcf), supernatant removed via magnetic assisted aspiration and resuspended in Milli-Q DI H₂O (4 mL) via ultrasonic sonication. More specifically, magnetic assisted aspiration was conducted via a 1 Tesla neodymium 1" cube magnet (CMS Magnetics, Plano, TX) placed at the bottom of the reaction vial in order to retain magnetic material in its pellet form during aspiration. These decorated particles were immediately used for the next step.

The final gold layer was fabricated by seed-mediated electroless plating. Decorated particles were vigorously mixed with a 1% HAuCl₄ –K₂CO₃ plating solution in a 1:10 ratio (thus 4 ml:40ml) . Briefly, K₂CO₃ (Sigma-Aldrich, 25 mg) was added to 100 mL of H₂O where 1% HAuCl₄ (dark-aged for 14 days prior) was added and dark-aged for 96 hours. H₂CO (Sigma-Aldrich, 400 µL) was added as a catalyst which began the release of Au ions thus causing a color change from clear to bright pink. Following a 30 min reaction time, particles were centrifuged (10 min, 800 rcf) and the supernatant was removed via magnetic assisted aspiration. Completed FeOx-SiO₂-Au nanoparticles were resuspended in DI H₂O (4 mL), thus quenching the plating solution, and stored at 4°C for further characterization. For storage longer than 10 days, the FeOx-SiO₂-Au nanoparticles were resuspended in 1.8 mM K₂CO₃ (4 mL) at 4°C. The FeOx-SiO₂-Au nanoparticles were measured to possess absorption in the NIR whereas gold nanoparticles do not have such absorptivity.

Formation of nanoparticles functionalized with antibody

FeOx-SiO₂-Au nanoparticles were conjugated with polyclonal antibodies specific for a target antigen by utilizing the following protocol. The exemplary antigen and antibody pair were recombinant human angiopoietin-2 (R&D Systems, 623-AN) and human angiopoietin-2 antibody (R&D Systems, AF623), respectively.

Antibody (90 µl, reconstituted to a concentration of 200 µg/ml in 1X filtered PBS) was mixed with a 9 µl solution of OPSS-PEG-NHS (Creative PEG Works, 3400 g/mol, 4.53 mg) dissolved in sodium bicarbonate (100 mM, 100ml) and stored at 4°C overnight. The antibody solution was then diluted to a volume of 1.8 ml and added to 1.8 ml of FeOx-SiO₂-Au nanoparticles at a concentration of $\sim 4.87 \times 10^9$ nanoparticles/ml. Following vigorous mixing, the solution was allowed to react for 1 hour at room temperature. Thereafter PEG-SH (Laysan Bio, Inc., 5000 MW, 38.48µM, 100µL) was added to the reaction and mixed vigorously, and stored at 4°C overnight to form a ~ 3.7 ml solution of FeOx-SiO₂-Au-antibody nanoparticles.

Formation of nanoparticle-antigen aggregates

FeOx-SiO₂-Au-antibody nanoparticles were aggregated with target antigen using the following protocol.

1.7 ml of 10X PBS was added to the ~ 3.7 ml solution of FeOx-SiO₂-Au-antibody nanoparticles. The target antigen (recombinant human angiopoietin-2 (R&D Systems, 623-AN)) was diluted to 2.2 µg/ml in 10X PBS. In a spectrophotometer, the absorbance protocol was zeroed by using a blank composed of 10X PBS (1.7 ml), sodium bicarbonate (1.9 ml), and DI H₂O (1.8 ml). 864 ml of FeOx-SiO₂-Au-antibody nanoparticles solution was added to a low volume cuvette and measured for a T=0

absorbance. Absorbance scans across a spectral range of 300-1100nm was measured for 30 minutes every 30 seconds. At T=60 seconds, 36 μ L of analyte was added to the solution. Progressive kinetic decrease was observed in the absorbance measurements over time. Such decrease in absorptivity of the nanoparticles solution is not seen in experiments using expired antigen solutions as a control. This decrease in absorbance supports the conclusion that the FeOx-SiO₂-Au-antibody nanoparticles form nanoparticle-antigen aggregates, and allow for the detection of the presence of the antigen.

Detecting the presence or concentration of quorum sensing proteins

Similar protocols will be used to detect quorum sensing proteins using nanoparticles functionalized with polyclonal antibodies raised against the quorum sensing proteins. The protocols also will be used as a correlative method for determining antigen concentration, a serial dilution creating a range of analyte concentrations (across a range of physiologically relevant analyte concentrations) can be performed which, upon repeated aggregation reactions, can generate a standard curve in accordance with Beer's law. The formula which represents this curve may be used to determine unknown analyte concentrations in biological media.

The methods and apparatus disclosure herein are not limited in their applications to the details of construction and the arrangement of components described herein. The invention is capable of other embodiments and of being practiced or of being carried out in various ways. Also it is to be understood that the phraseology and terminology used herein is for the purpose of description only, and should not be regarded as limiting. Ordinal indicators, such as first, second, and third,

as used in the description and the claims to refer to various structures, are not meant to be construed to indicate any specific structures, or any particular order or configuration to such structures. All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (e.g., "such as") provided herein, is intended merely to better illuminate the invention and does not pose a limitation on the scope of the invention unless otherwise claimed. No language in the specification, and no structures shown in the drawings, should be construed as indicating that any non-claimed element is essential to the practice of the invention.

Recitation of ranges of values herein are merely intended to serve as a shorthand method of referring individually to each separate value falling within the range, unless otherwise indicated herein, and each separate value is incorporated into the specification as if it were individually recited herein. For example, if a concentration range is stated as 1% to 50%, it is intended that values such as 2% to 40%, 10% to 30%, or 1% to 3%, etc., are expressly enumerated in this specification. These are only examples of what is specifically intended, and all possible combinations of numerical values between and including the lowest value and the highest value enumerated are to be considered to be expressly stated in this application.

Further, no admission is made that any reference, including any non-patent or patent document cited in this specification, constitutes prior art. In particular, it will be understood that, unless otherwise stated, reference to any document herein does not constitute an admission that any of these documents forms part of the common general knowledge in the art in the United States or in any other country. Any discussion of the

references states what their authors assert, and the applicant reserves the right to challenge the accuracy and pertinency of any of the documents cited herein.

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- [32] U.S. Patent No. 4,554,088
- [33] U.S. Patent No. 4,628,037
- [34] U.S. Patent No. 4,672,040
- [35] U.S. Patent No. 4,695,393
- [36] U.S. Patent No. 4,698,302

We Claim:

1. A composition for detecting the presence or concentration of a target antigen in a solution, comprising:

a plurality of nanoparticles, each comprising:

a core comprising iron oxide; and

a shell comprising at least one plasmon active metal;

wherein an exterior surface of each nanoparticle is functionalized with a plurality of polyclonal antibodies, each independently configured to bind to at least a portion of the target antigen;

wherein when a solution is prepared comprising the composition and the target antigen, at least some of the plurality of nanoparticles bind to the target antigen to form a nanoparticle-antigen aggregate;

wherein the nanoparticles that form the nanoparticle-antigen aggregate absorb electromagnetic radiation differently from nanoparticles that do not form the nanoparticle-antigen aggregate.

2. The composition of claim 1, wherein the nanoparticles that do not form the nanoparticle-antigen aggregate absorb electromagnetic radiation at a first peak wavelength, and the nanoparticles that form the nanoparticle-antigen aggregate absorb electromagnetic radiation at a second peak wavelength different from the first wavelength, and wherein at least one of the first peak wavelength and the second peak wavelength are in the near-infrared region or infrared region of the electromagnetic spectrum.

3. The composition of claim 2, wherein the first peak wavelength is less than the second peak wavelength.
4. The composition of any of claims 1-3, wherein the nanoparticles that do not form the nanoparticle-antigen aggregate have a molar extinction coefficient at a selected wavelength that is different from the molar extinction coefficient at the selected wavelength of the nanoparticles that form the nanoparticle-antigen aggregate.
5. The composition of any of claims 1-4, wherein the iron oxide comprises superparamagnetic iron oxide.
6. The composition of claim 5, wherein the superparamagnetic iron oxide comprises at least one of Fe_2O_3 , Fe_3O_4 , and a combination thereof.
7. The composition of any of claims 1-6, wherein at least one nanoparticle further comprises a first shell comprising a dielectric material at least partially surrounding the core, and a second shell comprising the least one plasmon active metal at least partially surrounding the first shell.
8. The composition of claim 7, wherein the dielectric material comprises SiO_2 .

9. The composition of any of claims 1-8, wherein the plasmon active metal comprises at least one of gold, silver, copper, platinum, and a combination thereof.
10. The composition of any of claims 1-9, wherein the exterior surface of at least one nanoparticle is functionalized by at least a first antibody configured to bind to a first portion of the target antigen, and a second antibody configured to bind to a second portion of the target antigen that is different from the first portion.
11. The composition of any of claims 1-10, wherein the target antigen is a bacterial quorum sensing protein.
12. The composition of any of claims 1-11, wherein the nanoparticles that do not form the nanoparticle-antigen aggregate have a first magnetic relaxation time, and the nanoparticles that form the nanoparticle-antigen aggregate have a second magnetic relaxation time different from the first magnetic relaxation time.
13. A device comprising a sample reservoir loaded with the composition of any one of claims 1-12.
14. The device of claim 13, further comprising a spectrophotometer and a receiver for receiving a cartridge comprising the sample reservoir.

15. A method of detecting the presence or concentration of a target antigen, comprising:

preparing a solution comprising the target antigen and a composition comprising a plurality of nanoparticles, each nanoparticle comprising:

a core comprising iron oxide; and

a shell comprising at least one plasmon active metal;

wherein an exterior surface of each nanoparticle is functionalized with a plurality of polyclonal antibodies, each independently configured to bind to at least a portion of the target antigen; and

wherein when the solution is prepared, at least some of the plurality of nanoparticles bind to the target antigen to form a nanoparticle-antigen aggregate; and

measuring a change in the absorbance spectra of the solution.

16. The composition of claim 15, wherein the nanoparticles that do not form the nanoparticle-antigen aggregate absorb electromagnetic radiation at a first peak wavelength, and the nanoparticles that form the nanoparticle-antigen aggregate absorb electromagnetic radiation at a second peak wavelength different from the first wavelength, and wherein at least one of the first peak wavelength and the second peak wavelength are in the near-infrared region or infrared region of the electromagnetic spectrum, and the measuring step comprises measuring a change in the absorbance of

near-infrared or infrared light at the first peak wavelength, the second peak wavelength, or both.

17. The method of claim 16, wherein the first peak wavelength is less than the second peak wavelength.

18. The method of any of claims 15-17, wherein the nanoparticles that do not form the nanoparticle-antigen aggregate have a molar extinction coefficient at a selected wavelength that is different from the molar extinction coefficient at the selected wavelength of the nanoparticles that form the nanoparticle-antigen aggregate.

19. The method of any of claims 15-18, wherein the iron oxide comprises superparamagnetic iron oxide.

20. The method of claim 19, wherein the superparamagnetic iron oxide comprises at least one of Fe_2O_3 , Fe_3O_4 , and a combination thereof.

21. The method of any of claims 15-20, wherein at least one nanoparticle further comprises a first shell comprising a dielectric material at least partially surrounding the core, and a second shell comprising the least one plasmon active metal at least partially surrounding the first shell.

22. The method of claim 21, wherein the dielectric material comprises SiO_2 .

23. The method of any of claims 15-22, wherein the plasmon active metal comprises at least one of gold, silver, copper, platinum, and a combination thereof.
24. The method of any of claims 15-23, wherein the exterior surface of at least one nanoparticle is functionalized by at least a first antibody configured to bind to a first portion of the target antigen, and a second antibody configured to bind to a second portion of the target antigen that is different from the first portion.
25. The method of any of claims 15-24, wherein the target antigen is a bacterial quorum sensing protein.
26. The method of any of claims 15-25, wherein the relative amount of the measured absorbance change is proportional to the concentration of antigen in the solution.
27. The method of any of claims 15-26, wherein the solution comprises a biological sample taken from a subject.
28. The method of claim 27, wherein the sample is selected from the group consisting of blood, saliva, sputum, urine, fecal matter, seminal fluid, vaginal fluid, and tissue.

29. The method of any of claims 15-28, wherein the nanoparticles that do not form the nanoparticle-antigen aggregate have a first magnetic relaxation time, and the nanoparticles that form the nanoparticle-antigen aggregate have a second magnetic relaxation time different from the first magnetic relaxation time, and wherein the method further comprising measuring a change in the magnetic relaxation time.

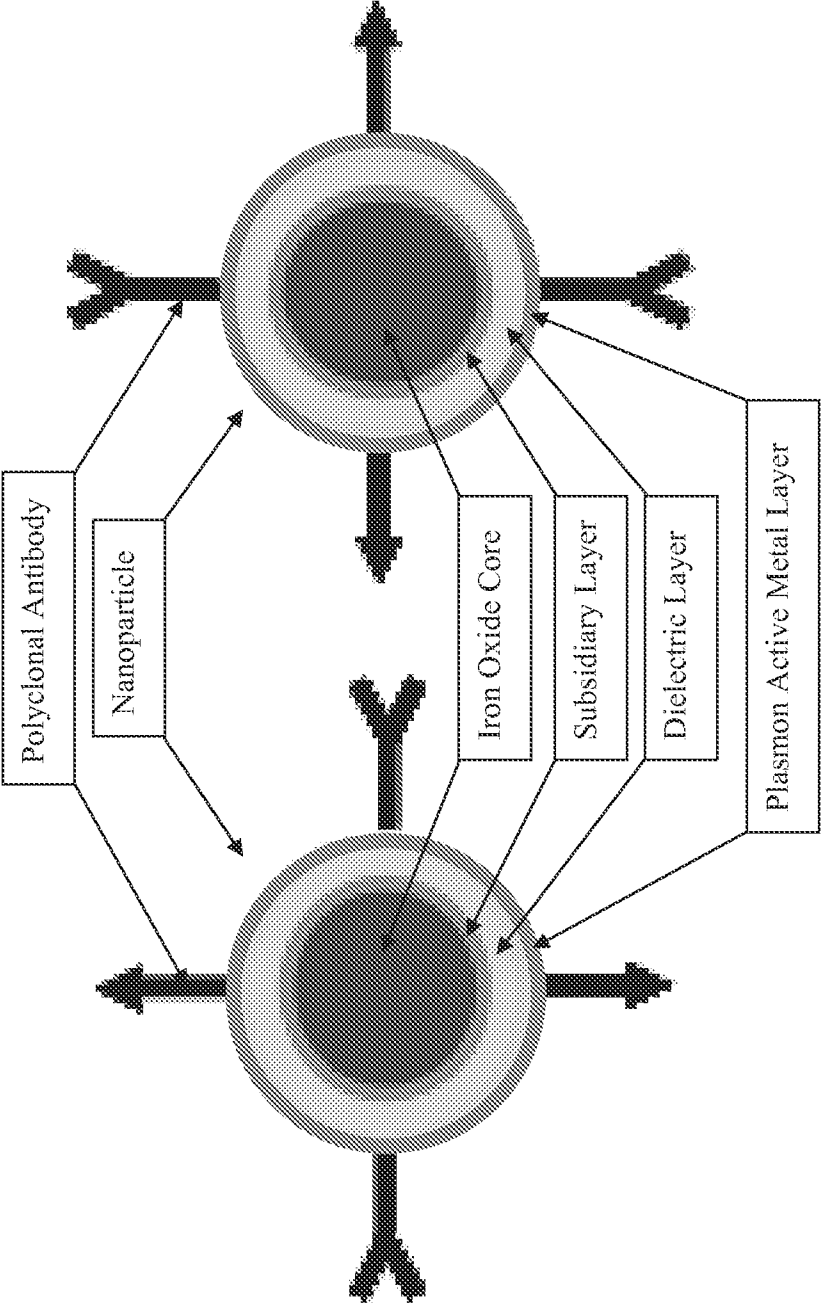


FIG. 1

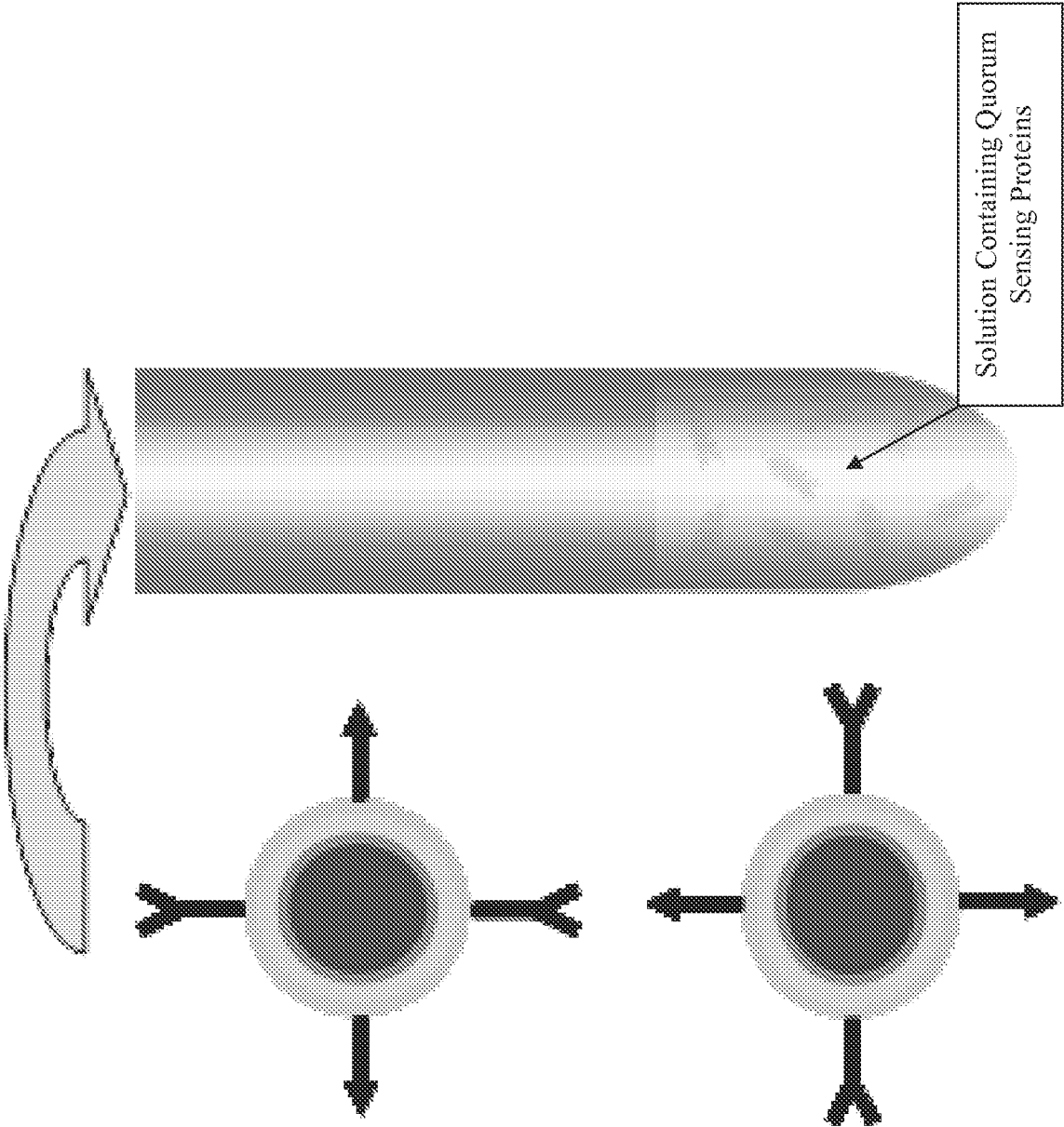


FIG. 2

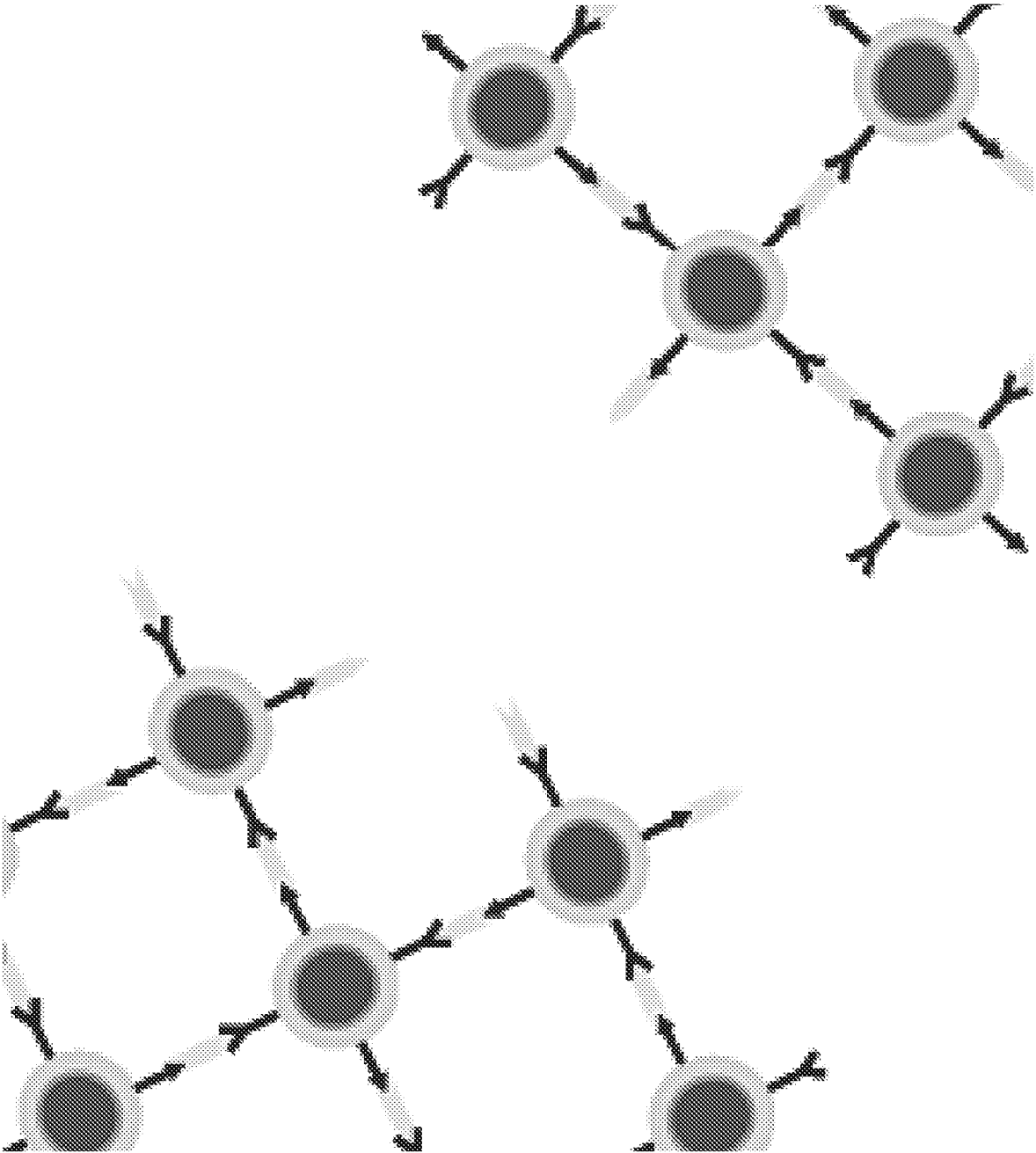


FIG. 3

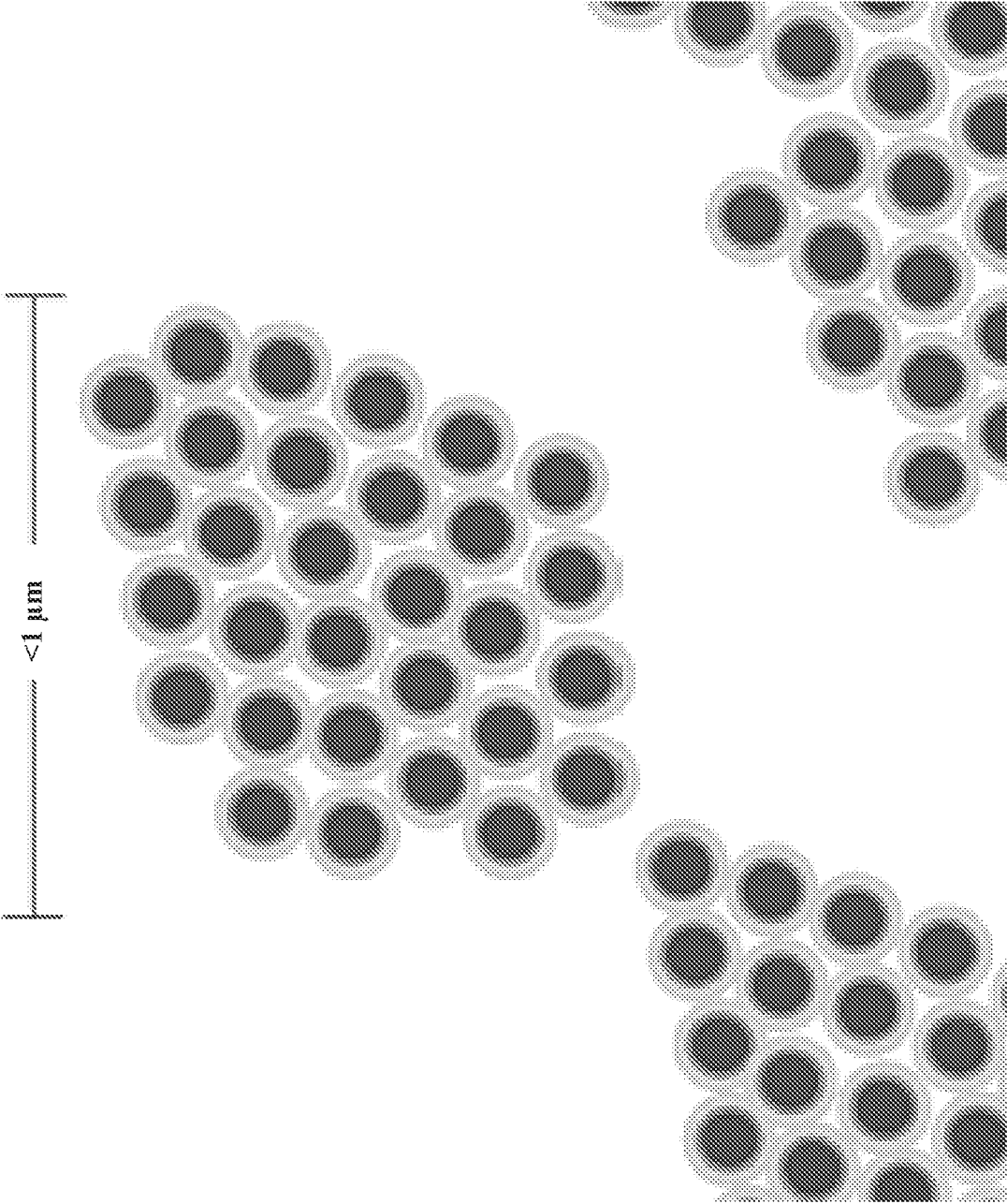


FIG. 4

INTERNATIONAL SEARCH REPORT

1013/040674 07-10-2013

International application No.

PCT/US13/40674

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - G01N 33/553, 33/51, 33/543 (2013.01)

USPC - 422/82.09; 435/7.1, 287.2

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC(8): G01N 33/553, 33/51, 33/543; B01D 15/38; C12Q 1/68, 1/25; C12P 1/00; B03C 1/01; B01J 20/06, 20/32 (2013.01)

USPC: 422/82.09; 435/7.1, 287.2, 971; 252/62.56; 428/405; 436/526, 525, 538, 541, 544, 806; 430/944

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

MicroPatent (US Granted, US Applications, EP-A, EP-B, WO, JP, DE-G, DE-A, DE-T, DE-U, GB-A, FR-A); Google; Google Scholar; DialogPro; iron oxide, Fe₂O₃, Fe₃O₄, core, nanoparticle, plasmon, metal, gold, silver, copper, platinum, coat, shell, layer, antibody, receptor, label, functional, antigen, ligand, target, assay, electromagnetic, infrared, near-infrared, IR, light, absorbance

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2008/140624 A2 (SOKOLOV, K et al.) November 20, 2008; figure 1B; paragraphs [0007]-[0009], [0018], [00173], [00185]	1-3, 4/1, 4/2, 4/3, 15-17, 18/15, 18/16, 18/17
X	US 2002/0177143 A1 (MIRKIN, CA et al.) November 28, 2002; entire document	1, 15
X	US 7801590 B2 (FELDMAN, MD et al.) September 21, 2010; entire document	1, 15
X	US 7700193 B2 (CHEN, MY et al.) April 20, 2010; entire document	1, 15
A	WO 2006/102307 A2 (KANG, KA et al.) September 28, 2006; entire document	1-4, 15-18
A	WO 2008/124525 A1 (SOMAN, CP et al.) October 16, 2008; entire document	1-4, 15-18
A	US 2009/0203154 A1 (CARTER, TJN et al.) August 13, 2009; entire document	1-4, 15-18
A	US 4698302 A (WHITEHEAD, RA et al.) October 6, 1987; entire document	1-4, 15-18
A	US 4554088 A (WHITEHEAD, RA et al.) November 19, 1985; entire document	1-4, 15-18

☐ Further documents are listed in the continuation of Box C.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

01 October 2013 (01.10.2013)

Date of mailing of the international search report

07 OCT 2013

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450

Facsimile No. 571-273-3201

Authorized officer:

Shane Thomas

PCT Helpdesk: 571-272-4300

PCT OSP: 571-272-7774

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: 5-14 and 19-29
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.